

Cryptosporidiosis in Ruminants: Update and Current Therapeutic Approaches

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Article history

Received: 11-04-2017

Revised: 29-05-2017

Accepted: 15-06-2017

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Abstract: *Cryptosporidium* species causes asymptomatic or mild to severe gastrointestinal disease in their broad range of host species. These host species include humans, domestic and wild animals worldwide. The importance of cryptosporidiosis in ruminants has been emphasised in recent years for not only mortality, delayed growth and economic losses in the farm but also zoonotic importance. So more than 200 substances have been tested against cryptosporidiosis, but none of them was able to eliminate the infection consistently. This article will focus on therapeutic interventions in neonatal ruminants against cryptosporidiosis including perspectives for new drugs.

Keywords: *Cryptosporidium*, Calf, Lamb, Kid, Treatment

Introduction

Cryptosporidium is an enteric protozoan parasite which causes gastrointestinal diseases on a different level. This species infects a wide range hosts including humans and animals worldwide. *Cryptosporidium* was first described in the laboratory mouse by Earnest Tyzzer in 1907. However, the clinical importance of this protozoan was not fully regarded in the medical and veterinary field for another 70 years (Tzipori and Ward, 2002). Bovine cryptosporidiosis was firstly documented in 1971 following identification of the parasites in an 8-month-old calf with chronic diarrhoea (Panciera *et al.*, 1971). Later, cryptosporidiosis was described in lambs with diarrhoea in Australia, but it could not be considered to be the cause of the disease because of concurrent pathogenic bacterial infection (Barker and Carbonell, 1974). Finally, in the early 1980s, studies on natural and experimental infections revealed the role of the protozoan in diarrhoea in ruminants as a primary etiological agent (Robertson *et al.*, 2013).

Cryptosporidium parvum affects many species of mammals that include human and it has considerable economic significance in farm animals, especially in the ruminant (Ryan and Hijjawi, 2015). Modern molecular methods are used to analyse the genetic profile of this parasite and new sub-species and genotypes of the parasite have been identified. Currently, 28 species have been recognised (Ryan *et al.*, 2014; Hotchkiss *et al.*, 2015). Different bovine species such as cattle, banteng, gayal, water buffalo and yaks were found susceptible to *C. parvum*, *C. bovis*, *C. andersoni* and *C. ryanae*. Also, small

ruminants are susceptible to *C. parvum*, *C. xiaoi* and *C. ubiquitosa*. It was reported that the primary source of the transmission of *C. parvum* to humans are ruminants such as cattle, goats and sheep and they have especially significant role in the human epidemics (Fayer, 1997; Thompson *et al.*, 2005; Shahiduzzaman and Dausgschies, 2012; Nasir *et al.*, 2013).

The life cycle of parasite consists of six major developmental phases which are excystation, merogony, gametogonia, fertilisation, oocyst wall formation and sporogony. Oocysts of *Cryptosporidium* spp. can sporulate within host cells and when they passed the feces becomes infective. In natural and experimentally induced cases in neonates, *C. parvum* not only affect the distal small intestine but also can affect proximal small intestine and some part of the large intestine (Laurent *et al.*, 1999; Constable, 2014). Some pathological changes can occur in affected animals intestine such as villus atrophy, villus degeneration, crypt hyperplasia, inflammatory infiltrations and microvillus degeneration. These changes cause maldigestion, malabsorption and osmotic imbalance (malabsorptive and secretor diarrhea) (Koudela and Jirí, 1997; McCole *et al.*, 2000; Gookin *et al.*, 2002; O'Handley and Olson, 2006). Because of all this, mild to moderate diarrhea its characteristic with watery, yellow or pale feces which contains mucus. Furthermore, the infected neonates can die in 3-4 weeks of life due to dehydration, weight loss, apathy, anorexia and energy deficit. On the other hand, Cryptosporidiosis is self-limiting in the neonates with strong immunity (Constable, 2014).

In experimentally *C. parvum* infected neonatal calves, it was demonstrated that there are two periods of oocyst shedding, as prepatent and patent, ranging from 3-6 and 4-13 days respectively. In practice, even in 3 days of age oocyst excretion has been demonstrated. Which means, infection susceptibility presents immediately after birth of the calves. Oscillation of the prepatent period in lambs and goat kids is between 2-7 days and around 4 days, respectively. However, even the severity of clinical signs is reduced, the animal's susceptibility to infection continues in further age (de Graaf *et al.*, 1999).

Because of its mortality rate, growth and developmental delaying effect and other reasons, Cryptosporidiosis is a disease with economic importance. Also, livestock, particular cattle are one of the most important reservoirs of zoonotic infections. The factors contribute the epidemiology of *Cryptosporidium* are extraction of the oocysts in large quantities, survival of the oocysts in warm and moist conditions for months, arising from the infection can be caused by a small number of oocysts, even from a single oocyst for susceptible individuals (de Graaf *et al.*, 1999; Ryan *et al.*, 2014).

Cryptosporidiosis is a major problem for farm and public health since there are no effective treatment and prevention methods although many substances have been tested, no agent has been found to control or eliminate the cryptosporidiosis consistently. Only, oocyst shedding can be reduced by using some medicines. For effective control of infection, improving hygienic conditions and drug applications should be carried out in combination because no measure is sufficient alone against to cryptosporidiosis (de Graaf *et al.*, 1999; Shahiduzzaman and Dauschies, 2012). New treatment approach such as drug treatment and alternative therapy options will be addressed in this review.

Drug Treatment for Cryptosporidiosis in Ruminants

Treatment of cryptosporidiosis in farms falls broadly into three categories: Anti-Cryptosporidial drug therapy, supportive therapy and reduction of environmental oocyst load. One or more of these therapeutic options are used for controlling the infection. Mostly, anti-cryptosporidial agent trials were performed on laboratory animals. Although there are many promising agents *in vitro* or *in vivo* models, none of these agents is useful for the elimination of cryptosporidiosis from the infected animal. Some conditions such as the immune status of the animals, concomitant other infectious agents and effective supportive treatment affect the clinical and parasitological responses to treatment. However, drugs that have been applied with more or less efficiency reviewed here for ruminants (Shahiduzzaman and Dauschies, 2012).

Azithromycin

Azithromycin is a macrolide antibiotic, which has variable efficiency and has been used for the treatment of cryptosporidiosis of immune suppressed animals and humans (Rehg, 1991; Holmberg *et al.*, 1998). Azithromycin shows the antibacterial mode of action by inhibition of protein synthesis (Armson *et al.*, 2003).

Elitok *et al.* (2005) randomised 50 naturally infected calves with *C. parvum* and medicated orally with azithromycin at the doses of 500, 1000, 1500 and 2,000 mg PO once daily for 7 days. On days 14 and 21 following the cessation of the drug application calves were monitored in terms of drug efficiency. The most effective doses for reduction of oocyst count was found as 1,500 and 2,000 mg d⁻¹. Nasir *et al.* (2013) and Kumar *et al.* (2015) reported that Azithromycin (1500 mg day⁻¹ for 7 days) therapy of cryptosporidiosis caused significant clinical improvement and reduction of oocyst count in naturally infected calves.

Despite above these studies, novel data from clinical trials aren't sufficient for control of cryptosporidiosis in calves. Because these studies were performed field conditions and animals may be re-infection. Also, Azithromycin is expensive in many countries so it may not be economical for farms (Shahiduzzaman and Dauschies, 2012).

Paromomycin

Paromomycin is an aminoglycoside and most of the medicine is removed from the stool in oral usage because of its poor absorption from the intestine (Johnson *et al.*, 2000).

Grinberg *et al.* (2002), reported a significant reduction in stool frequency and oocyst excretion after application of paromomycin at 100 mg kg⁻¹ orally, once a day for 10 days, but oocysts excretion and diarrhoea started following cessation of the drug in naturally infected calves. Also, Masood *et al.* (2013) reported that paromomycin (25 mg kg⁻¹ BW, for 5 days) reduced quantity and duration of oocyst shedding in experimentally infected calves.

A previous study indicated that paromomycin (25 mg kg⁻¹) significantly decreased to faecal oocyst count starting from a post treatment 3rd day in naturally infected kids and lambs (Shafiq *et al.*, 2015). Also, Viu *et al.* (2000) indicated that paromomycin application to lambs at 100 mg kg⁻¹ BW per day for three consecutive days or 200 mg kg⁻¹ BW per day for two days reduced both oocyst shedding and severity of diarrhoea.

Mancassola *et al.* (1995) used treated the experimentally infected goats by using paromomycin and observed that antibodies to *Cryptosporidium parvum* developed. Besides, the increase of antibody was associated with protection and prevention of oocyst shedding.

In a comparative study, a single dose of 50 mg kg⁻¹ body weight of metronidazole caused a significant decrease in oocyst count per gram faeces from 3rd-day post treatment. Moreover, Metronidazole was found more effective than paromomycin in naturally infected lambs and kids (Shafiq *et al.*, 2015). On the other hand, Masood *et al.* (2013) reported that Paromomycin to be the more valuable than metronidazole for the treatment of *Cryptosporidium* infection in cattle based on the clinical trials.

The effect of Paromomycin on the reduction of clinical signs and oocyst excretion does not seem consistent although the data are promising. (Ritchie and Becker, 1994). Also, of major concern in the testing of the clinical efficacy of any drug are its toxic effects. Paromomycin is an aminoglycoside and so might cause nephrotoxicity and ototoxicity (Johnson *et al.*, 2000). This product is not used in ruminants generally.

Nitazoxanide

Nitazoxanide (NTZ) is an antimicrobial substance that has been applied for antiparasitic treatment in dogs, cats, sheep and goats (Euzeby *et al.*, 1980; Gookin *et al.*, 2001; Viel *et al.*, 2007). Additionally, NTZ has been FDA approved for human use for treatment of cryptosporidiosis.

Sahu and Maiti (2015) reported that NTZ administered to calves at 15 mg kg⁻¹ BW twice a day for three consecutive days reduced both oocyst shedding and severity of diarrhoea as reported by Viel *et al.* (2007) in experimentally infected goats. On the other hand, Schnyder *et al.* (2009) reported that the effect of NTZ on clinical severity or oocyst excretion in the experimentally *Cryptosporidium* infection was less than expected.

Treatment efficiency of NTZ is controversial. However, it has consistent side effects such as stomachache, headache and emesis. The oral administration of NTZ to calves (15 mg kg⁻¹ BW, BID) affected the mucous membranes of the gastrointestinal tract. Similar effects were also observed in piglets (Theodos *et al.*, 1998), cats (Gookin *et al.*, 2001) and neonatal ruminants (Viel *et al.*, 2007; Schnyder *et al.*, 2009).

Oral administration at a dose of 75-400 mg kg⁻¹ BW in the sheep caused to mild diarrhea that lasts for about 24-36 h and mild catarrhal inflammation. (Euzeby *et al.*, 1980) Therefore, it is not recommended for either treatment or prophylaxis of cryptosporidiosis (Shahiduzzaman and Dausgchies, 2012).

Halofuginone Lactate

Halofuginone lactate which is a synthetic product of the quinazolinone group with antiprotozoal activity, has received positive reports from studies related to the treatment of Cryptosporidiosis in veal calves (Lallemond *et al.*, 2006) dairy calves (Villacorta *et al.*,

1991; Klein, 2008) and lambs (Naciri and Yvore, 1989). Clinical studies showed that halofuginone had cryptosporidiastatic activity in the sporozoite and merozoite stages of *C. parvum* (Jarvie *et al.*, 2005). Because of halofuginon delays the onset of infection decreases oocyst shedding and cryptosporidiosis severity, it has been recommended for both therapeutic and prophylactic use (Joachim *et al.*, 2003; Jarvie *et al.*, 2005).

According to Weber *et al.* (2016), halofuginone is approved for prophylaxis against cryptosporidiosis, but it showed no effect on the excretion of *Cryptosporidium* oocysts in calves. Conversely, there are different studies which halofuginone showed no effect on the course of infection concerning diarrhoea and dehydration. (Lallemond *et al.*, 2006) alternatively, seven days after the drug was withdrawn, oocysts appeared again in faeces, indicating re-infection (Shahiduzzaman and Dausgchies, 2012).

In general, ruminants were cured with 0,1-0,12 mg kg⁻¹ BW orally for 7 days. Also, halofuginone at the recommended dose (0,1 mg kg⁻¹ for seven consecutive days) seems to restraints cryptosporidial reproduction rate and improves the development of immunity in lambs (Causape *et al.*, 1999; Giadinis *et al.*, 2007).

According to Chartier *et al.* (1999), the administration of halofuginone to 3-day old kids for 7-10 days decreased the prevalence and intensity of *Cryptosporidium* oocyst output when compared to control, particularly from 3 to 7 following the start of drug administration. Similarly, Giadinis *et al.* (2008) showed a reduction of oocyst shedding on days 5 and 7 following the beginning of administration at 1-2 day of age in kids.

Also, Petermann *et al.* (2014) reported that when given as a prophylactic treatment at 0,1 mg kg⁻¹ BW during 10 days in reducing oocyst shedding, diarrhoea and mortality in goat kid affected by *C. parvum*.

This drug is approved in Europe to treat calf cryptosporidiosis. The disadvantage of this product is that it must be given orally for 7 days from birth and this is not always practical, especially in beef suckler units. Also, it is vitally important that farmers be aware of the potential effects of overdosing as toxic effects have been shown at only twice the recommended dose. The product is also licensed for the treatment of diarrhoea due to cryptosporidiosis however it must not be given to dehydrated or inappetence animals (Robertson *et al.*, 2013).

Decoquinatate

Decoquinatate is quinolone coccidiostat that found to have little activity against *C. parvum in vitro* or *in vivo* in suckling mice (Lindsay *et al.*, 2000).

Redman and Fox (1993) found that decoquinatate reduced the shedding of oocysts and the clinical signs in experimentally induced cryptosporidiosis in calves when

decoquinate is used at 2 mg kg⁻¹ BW dose for 21 days. In contrast, decoquinate had no effect either on the shedding of oocysts or on the calves' clinical signs, in agreement with the results of Moore *et al.* (2003).

In kids, the decoquinate at 2,5 mg kg⁻¹/day for 21 days, although it did not eradicate cryptosporidiosis, it gave significant improvements, no diarrhoea and decrease in oocyst shedding (Mancassola *et al.*, 1997).

Decoquinate is commonly used by producers to prevent cryptosporidiosis (Moore *et al.*, 2003) but the practice should be reconsidered on account of its questionable efficacy.

Alternative Therapy for Cryptosporidiosis in Ruminants

The studies for alternative therapy which could be used as the anti-parasitic treatment had received considerable attention in recent years because of the increasing worldwide development of resistance to the synthetic drug in parasitic populations.

Activated Charcoal and Wood Vinegar

Activated charcoal can bind with various molecules and because of this, it is known as a universal adsorbent (Chandy and Sharma, 1998). For the removal of bacteria and bacterial toxins both *in vitro* and *in vivo*, it was reported that activated charcoal could be used effectively (Drucker *et al.*, 1977; Pegues *et al.*, 1979; Du *et al.*, 1987; Gardiner *et al.*, 1993).

In a study on experimentally infected calves, rapid clinical improvement and cessation of oocyst excretion revealed after a short time following treatment of activated charcoal that contains wood vinegar liquid (Watarai *et al.*, 2008). These authors also set up *in vitro* experiments to explain the activity of the 2 components of the product. They observed that activated charcoal adsorbed *C. parvum* oocysts and that the number of *C. parvum* oocysts was lowered by the addition of increasing concentrations of wood vinegar.

In another study conducted in field condition, a product containing activated charcoal and wood vinegar liquid was given to goat kids at the dose rate of 3.75 g/day/kid (Obionekk®) for defensive purposes. It was administered as a suspension in milk replacer twice or three times a day starting at the age of 3 days to the age of 17 days. Compared with the control group, oocyst excretion was significantly reduced when the product was given 3 times a day and clinical signs also significantly resolved whatever the rhythm of distribution (Paraud *et al.*, 2011).

Consequently, this product may be helpful for the treatment and prevention of cryptosporidiosis in ruminants but it does not eradicate the disease.

Nigella Sativa

Nigella sativa seeds have been known with wide therapeutic effects. Its positive effects against skin diseases, jaundice, gastrointestinal problems, anorexia, conjunctivitis, dyspepsia, rheumatism, diabetes, hypertension, intrinsic haemorrhage, paralysis, amenorrhoea, anorexia, asthma, cough, bronchitis, headache, fever, influenza and eczema have been reported (Forouzanfar *et al.*, 2014).

Nigella sativa has been reported to have good anti-coccidial activity with no harmful side effects in rabbits at a dose rate of 400 mg kg⁻¹ BW orally for 14 days (Baghdadi and Al-Mathal, 2011). In a study by Nasir *et al.* (2013) on the therapeutic efficacy of *Nigella Sativa* showed no effect on oocyst shedding in naturally infected dairy calves. Studies on the efficacy of *Nigella Sativa* against *C. parvum* are rare, but it has been shown to be effective as antihelminthic and anticoccidial (Nasir *et al.*, 2013).

Clinoptilolite (Zeolite)

Clinoptilolite which is a natural zeolite is mineral mined from sedimentary deposits (Leung *et al.*, 2007). Zeolites retain moisture due to the desorption of loosely bound water present on, or close to the external zeolite surface (Leggo *et al.*, 2006). Clinoptilolite could modify appropriate environmental conditions of humidity for oocyst to sporulate and become infective and also it has got the immunomodulator effect for the host (Jurkic *et al.*, 2013).

Ulutas *et al.* (2015) reported that in neonatal lambs infected with *C. parvum* that were receiving prophylactic clinoptilolite the appearance of diarrhea as well as duration and intensity of oocyte shedding was considerably reduced. In addition, the severity of cryptosporidiosis was reduced in the clinoptilolite group.

Garlic (Allium Sativum-Garlicin)

As an extract of garlic, garlicin has been confirmed to have multiple curative effects such as antimicrobial, antithrombotic, hypolipidemic and antitumor activities. Lately, some reports tested the effect of garlicin in the treatment of parasites such as toxoplasmosis, trichomoniasis and cryptosporidiosis (Huang *et al.*, 2015).

Garlic was a convenient prophylactic and a promising therapeutic agent for cryptosporidiosis (Abdel Megeed *et al.*, 2015). The efficacy of garlic in the prophylaxis and treatment of experimental cryptosporidiosis could be explained by a different mechanism. El-Shenawy and Hassan (2008) reported the enhancement of phagocytosis and an increase in natural killer cell activity which promoted the immune system function and strengthened the body's defence mechanism during the duration of treatment by garlic. Moreover, Masamha *et al.* (2010) found that *Allium*

sativum disrupted the normal physiological functions of the parasite like morbidity and reproduction.

In a study by Abdel Megeed *et al.* (2015) on the therapeutic efficacy in naturally infected Buffalo calves of garlic showed it eradicated the *Cryptosporidium* oocysts from the faeces and almost no oocysts were detected in the faeces of garlic treatment group by the end of the study. Also, the same study showed that garlic was effective not only in reducing oocyst counts but also in making the health of the animals better.

Garlic offered a promising effect in the treatment of cryptosporidiosis and it is recommended to be used in the daily animal diet to give protective effects against parasites (Abdel Megeed *et al.*, 2015).

Conclusion

Cryptosporidiosis is a parasitic intestinal disease of a wide variety of animals and humans. Major reservoirs of *C. parvum* are livestock, especially cattle and sheep. Transmission of the infection may be directly from animal to human or indirectly via a vehicle. A large number of small outbreaks associated with *C. parvum* in calves and in veterinarians or veterinary students that have been exposed to calf faeces are documented in the literature (Gait *et al.*, 2008; Grinberg *et al.*, 2011; Robertson *et al.*, 2013). Most indirect transmission is drinking water and less often food has been associated with transmission of *Cryptosporidium* infection from animals to human populations. A birth cohort study conducted by a Global Network for the Study of Malnutrition and Enteric Diseases (MAL-ED) has assessed pathogen-specific burdens in diarrhoeal and non-diarrhoeal stool specimens from 2145 children aged 0-24 months in Africa, Asia and South America and also identified *Cryptosporidium* spp. as one of the five highest attributable burdens of diarrhoea in the first year of life (Platts-Mills *et al.*, 2015; Ryan *et al.*, 2016). Globally, Cryptosporidiosis is estimated to be responsible for the majority of deaths among children under 5 years of age (Ryan *et al.*, 2016).

The importance of cryptosporidiosis in ruminants has been emphasised in recent years for not only mortality, delayed growth and economic losses in the farm but also zoonotic importance. The "One Health" approach to tackling the zoonotic disease, defined as "One Medicine" by Schwabe (1969), is a worldwide strategy to improve health and well-being through the mitigation and prevention of infection. Despite exhaustive attempts at chemotherapy, no effective treatment for cryptosporidiosis had been identified (Abdel Megeed *et al.*, 2015). For example, halofuginone is licensed for the treatment of bovine cryptosporidiosis in several countries, but it is known to be relatively toxic and care must be taken not to exceed the claimed dose (Shahiduzzaman and Dausgies, 2012). Because of the limited availability of effective

drugs, sanitary measures and good management are currently the most valid weapons in controlling this disease.

In conclusion, funding has recently been secured to study more closely the host-parasite interaction with the neonatal gut, to better understand the early response to infection. The new studies focused on mainly genetic characterization and protein analyses of the parasite. Bioinformatic and proteomics studies have recently gained increased recognition for this purpose. These studies will open the way for a more careful approach to chemotherapy for cryptosporidiosis in the near future. Thus it is the author's advice that primary aim at controlling the cryptosporidiosis in farms should be minimising the build-up of environmental contamination and reducing the clinical impact by optimising the neonatal environment and managing other enter pathogens until effective drugs are developed.

Acknowledgement

Any financial support was used to write this manuscript.

Author's Contributions

Ceren Dinler: Carried out literature research, writing and draft the review.

Bulent Ulutas: Supervision of the review, critical review and organise the manuscript period.

Ethics

This review is original and was not published elsewhere. It can be confirmed that all authors have read and approved the manuscript and no ethical issues involved.

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